Beagle puppy model of perinatal cerebral infarction

Acute changes in cerebral blood flow and metabolism during hemorrhagic hypotension

LAURA R. MENT, M.D., WILLIAM B. STEWART, PH.D., CHARLES C. DUNCAN, M.D., BRUCE R. PITR, PH.D., ALDO RESCIGNO, PH.D., AND JUDY COLE, M.S.

Departments of Pediatrics, Neurology, Neurosurgery, Gross Anatomy, and Anesthesia, Yale University School of Medicine, New Haven, Connecticut

Asphyxia, with its attendant hypotension, is by far the most common cause of neonatal cerebral infarction and frequently results in lesions of the parieto-occipital white matter. This study examines the effects of hypotension on regional cerebral blood flow (CBF), local cerebral glucose utilization (LCGU), and serum prostaglandin levels in newborn beagle pups. The animals (24 to 96 hours old) were anesthetized, tracheotomized, and paralyzed. Pups were randomly divided into two groups: one was subjected to hemorrhagic hypotension and the other received no insult. Hypotension was induced by slow venous hemorrhage to maintain a mean arterial blood pressure of 20 to 30 mm Hg. Autoradiographic determinations of LCGU using carbon-14 (\(^{14}\)C)-2-deoxyglucose were performed 45 minutes after randomization to groups. Autoradiographic determinations of CBF were performed using \(^{14}\)C-iodoantipyrine on a second group of pups 15 minutes after randomization. Prostaglandins were measured immediately before and 15 minutes after insult or control manipulation. There were no significant differences in the values for thromboxane B\(_2\) or 6-keto-prostaglandin F\(_{1\alpha}\), the stable breakdown products of thromboxane A\(_2\), and prostacyclin. Prostaglandin E\(_2\) levels significantly increased in response to hemorrhagic hypotensive insult. In addition, although regional CBF was maintained in cortical and central gray matter structures during hypotension, CBF to the periventricular temporal and parietal white matter zones significantly decreased, and LCGU was increased in these same regions during hypotensive insult. The uncoupling of CBF and metabolism in these periventricular white matter regions may be responsible for the neuropathological sequelae of perinatal asphyxia.

KEY WORDS • neonatal brain • beagle puppy model • hemorrhagic hypotension • cerebral blood flow • metabolism

In most recent series, perinatal asphyxia, with its attendant hypotension, appears to be the most frequently determined etiology for stroke,\(^{15,30,31}\) and the neurodevelopmental outcome in these infants remains worrisome.\(^{28,42}\) The newborn beagle pup has been demonstrated to provide a good model for the study of neonatal cerebral pathophysiology.\(^{23}\) Neuropathological insults similar to those found in newborn infants may be made using clinically relevant models, and studies of cerebral blood flow (CBF) and metabolism can be undertaken.\(^{35,36}\) The newborn beagle pup has been used in carbon-14 (\(^{14}\)C) autoradiographic studies of the effects of hemorrhagic hypotension on CBF and metabolism in the developing brain. Results of these studies are presented in this article.
Materials and Methods

Preparation

Studies were performed on two separate groups of newborn beagle pups. The first group of 12 animals underwent determinations of CBF and the second group of 18 pups underwent determinations of local cerebral glucose utilization (LCGU). Both groups of pups underwent similar physiological preparation and monitoring, as well as similar experimental protocols for prostaglandin determinations and the induction of hemorrhagic hypotension.

Newborn beagle pups (24 to 96 hours old) were anesthetized with intraperitoneal pentobarbital and tracheotomized under local anesthesia (1% xylocaine). They were paralyzed with subcutaneous pancuronium bromide (1 mg/kg) and ventilated with a mixture of 30% oxygen and 70% nitrous oxide (for analgesia). Under local anesthesia, bilateral femoral venous and arterial lines were inserted by cutdown procedures. Arterial blood pressure was monitored utilizing a pressure transducer and polygraph recorder. Body temperature was recorded by a thermal probe and maintained by means of a warming light at 36.5°C to 37.5°C. Ventilation rate and total volume were adjusted to maintain arterial normoxia (40 to 60 torr) and normocapnia (30 to 40 torr). When the pups were physiologically stabilized, 1 ml of blood was withdrawn from the femoral venous catheter for the first prostaglandin determination. Following this procedure, the pups were randomly assigned to a hemorrhagic hypotension group or a control group.

Hypotension was induced by slow withdrawal (1 ml/min) of blood from the femoral venous catheter until the mean arterial blood pressure (MABP) reached severe hypotension (MABP 20 to 30 torr). Pups in the control group did not undergo withdrawal of blood but were observed for a comparable experimental period of time.

Immediately following random assignment to hemorrhagic hypotension or no-insult groups, those animals in which metabolism was to be studied underwent 14C-2-deoxyglucose (2DG) determinations of LCGU. Fifteen minutes after randomization, all animals underwent the second prostaglandin determination. Immediately following this, 14C-iodoantipyrine measurements of CBF were begun in the group for CBF study, and the animals were rapidly sacrificed thereafter. Animals undergoing LCGU determinations were sacrificed following 45 minutes of hypotension. The CBF determinations were made at 15 minutes to correspond to the period of greatest cerebral uptake of 2DG for LCGU.

Cerebral Blood Flow Studies

The CBF determinations were made under continued anesthesia by a fast intravenous infusion of 50 µCi 14C-iodoantipyrine simultaneously with the rapid arterial withdrawal of blood into an artificial organ system composed of approximately 30 cm of polyethylene tubing (PE-60) attached to a Harvard infusion/withdrawal pump* calibrated to withdraw blood at a constant rate of 2.72 ml/min, as described by Cavazzuti and Duffy.† At the end of this 5-second period, the animals were rapidly decapitated and the brains were removed and placed in isopentane chilled to −60°C. Brain sections 32-µ thick were prepared with a cryostat maintained at −15°C to −10°C, and every 25th section was placed on a glass slide, dried on a hot plate at 60°C to 70°C, and placed sequentially in an x-ray cassette loaded with Kodak SB-5 film for 7 days. Calibrated plastic standards with known concentrations of 14C were placed adjacent to the tissue sections.

Local tissue concentrations were determined by densitometric measurements.† Values were obtained bilaterally from six cortical sites, six white matter sites, three caudate nucleus regions, and two germinal matrix sites. Arterial blood withdrawn during the 14C-iodoantipyrine intravenous injection was placed in preweighed scintillation vials, and an aliquot of blood was removed in triplicate and treated with an equal volume of hydrogen peroxide. The 14C determinations were made using a Packard 4550 scintillation counter‡ and standard liquid spectrometry.

The following formulation was used to determine CBF values (ml/100 gm/min):

\[
\text{CBF} = \frac{\mu \text{Ci in brain/gm}(2.22 \times 10^3 \text{ dpm/} \mu \text{Ci})(2.72 \text{ ml/min})}{(\text{total dpm in syringe blood})}
\]

where dpm = disintegrations per minute.

Local Cerebral Glucose Utilization Studies

The LCGU was determined in the pups according to the methods of Duffy, et al.,11 during a 45-minute period of hypotension, as described above. When the pups were physiologically stable, a 0.5- to 0.75-ml bolus dose of 2DG (0.2 mCi/kg body weight in saline) was injected intravenously via the femoral venous line at time 0. Prior to and at intervals of 1, 2, 3, 4, 7, 10, 15, 25, 35, and 45 minutes following the injection, 75 µl of blood was collected through the femoral arterial line into tubes containing heparinized bilirubin and centrifuged to separate the plasma. After 45 minutes, the animals were rapidly sacrificed; the brains were removed and prepared for 14C autoradiography as described for the CBF studies. The glucose content of the arterial samples was determined by means of a Beckman glucose analyzer.§ Activity of 14C was similarly determined by means of a Beckman glucose analyzer.§

† Leitz microdensitometer manufactured by E. Leitz, Inc., Rockleigh, New Jersey.
‡ Packard 4550 scintillation counter manufactured by Packard Instrument Co., Inc., Downers Grove, Illinois.
§ Beckman glucose analyzer manufactured by Beckman Instruments, Inc., Fullerton, California.
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determined on the arterial plasma samples by adding 10 μl of plasma to 1 ml water and 15 ml Omniflor-dioxane in scintillation vials and counting in a liquid scintillation counter.

Autoradiographs were processed and regional 14C concentrations determined as described for the CBF studies. The LCGU (in μmol/gm/min) was calculated from the total tissue 14C activity at the time of killing, as determined autoradiographically (C*(T)), and from the concentrations of glucose (Cp) and 2DG (C*) in arterial plasma, according to the operational equation:

$$\text{LCGU} = \frac{C^* (T) - k^* e^{-k^* t} \int_0^T C^* e^{k^* t} dt}{\left( \frac{V^*}{V_m} K_m \right) \int_0^T C^* (C_p) e^{-k^* t} dt}$$

where k* denotes the rate constant for 2DG transport into brain and (k* + kס) is the rate constant for 2DG turnover in the brain, K* and Kм are Michaelis-Menten constants, and V* and Vм are the maximal velocities of hexokinase for deoxyglucose and glucose, respectively. The following values for these rate constants have been previously determined in the adult rat 44 and utilized by Duffy, et al., for determinations of LCGU in hypoxemic newborn beagle pups: gray matter: k* = 0.189, kס = 0.245, kש = 0.052; white matter: k* = 0.079, kס = 0.133, kש = 0.020. Although uncertainty about the true values of these rate constants may introduce some error, the magnitude of this error is believed to be small since all of the terms in the equation that contains these rate constants approach zero with increasing time after the radioisotope is injected, and because we carried out our measurements for 45 minutes (nine half-lives of the turnover of free 2DG).

The value for the lumped constant to be used in these calculations is that determined by Duffy, et al., in the newborn beagle puppy brain (0.558).

Prostaglandin Determinations

Blood drawn for measurement of prostaglandin (PG) levels was placed into chilled glass tubes containing ethylenediaminetetra-acetic acid (EDTA) and centrifuged for 10 minutes (4°C at 2400 G); the plasma was stored at −70°C. Aliquots of plasma were assayed in triplicate for the determination of thromboxane (TX) B2 (TXB2), 6-keto-PGF1α, and PGE2. The first is the stable breakdown product of TXA2, and the second is the stable metabolite of prostacyclin, PGI2. Assays were performed utilizing radioimmunoassay kits.*

**Pairwise and unpaired t-tests were used for statistical analysis of the data collected.

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Omniflor-dioxane obtained from New England Nuclear Corp., Cambridge, Massachusetts.

Radioimmunoassay kits obtained from New England Nuclear Corp., Cambridge, Massachusetts.

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TABLE 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CBF Series</th>
<th>LCGU Series</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of animals</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.04</td>
<td>7.37 ± 0.05</td>
</tr>
<tr>
<td>pO2 (mm Hg)</td>
<td>57 ± 4</td>
<td>56 ± 6</td>
</tr>
<tr>
<td>pCO2 (mm Hg)</td>
<td>34 ± 3</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>body weight (gm)</td>
<td>380 ± 15</td>
<td>290 ± 15</td>
</tr>
</tbody>
</table>

* Mean values ± standard deviation. CBF = cerebral blood flow measurement; LCGU = local cerebral glucose utilization determination.

TABLE 2

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>CBF Series</th>
<th>LCGU Series</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>72 ± 6</td>
<td>71 ± 8</td>
</tr>
<tr>
<td>hemorrhagic hypotension</td>
<td>73 ± 8</td>
<td>74 ± 5</td>
</tr>
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</table>

* Mean values ± standard deviation at 0 and 15 minutes after insult or control group preparation. CBF = cerebral blood flow measurement; LCGU = local cerebral glucose utilization determination.

Results

Data are available for 12 animals for the CBF studies and for the 18 pups who underwent 14C-2DG determinations of LCGU.

Cerebral Blood Flow Determinations

The 14C-iodoantipyrine determinations of CBF were performed on 12 pups, six of which had been subjected to hemorrhagic hypotension. Arterial blood gas values and animal weights are found in Table 1; no significant differences in any of these parameters were noted.

The MABP of the control pups, as shown in Table 2, was 72 ± 6 mm Hg throughout the experimental period. The MABP of the animals exposed to hemorrhagic hypotension was 73 ± 8 mm Hg before initiation of the insult and 24 ± 3 mm Hg 15 minutes after. Prostaglandin data are not available for the CBF series of experiments.

Cerebral blood flow values are found in Table 3. For the control pups, CBF values were 17.4, 20.5, and 15.4 ml/100 gm/min, respectively, for the frontal, temporal, and parietal gray matter regions. In the hemorrhagic hypotension animals, values of 13.9, 14.8, and 16.9 ml/100 gm/min were found for the same regions. The CBF values for the periventricular white matter were 4.7, 6.0, and 5.7 ml/100 gm/min for the frontal, temporal, and parietal regions, respectively, compared to 3.0, 2.2, and 1.8 ml/100 gm/min for the same regions in animals exposed to hemorrhagic hypotension insult.
As shown in Table 3, there were significant decreases (p < 0.01) in CBF to both the temporal and parietal white matter of pups exposed to insult. No difference was noted in CBF to the caudate nucleus region of the control animals when compared with the experimental group.

**Studies of Local Cerebral Glucose Utilization**

Determination of LCGU were successfully performed on 18 pups, 10 of which had been subjected to hemorrhagic hypotension insult. As shown in Table 1, there were no significant differences in arterial blood gas values, body weights, or plasma glucose values for the two groups of animals undergoing LCGU study. The MABP values for the control pups and the experimental animals are found in Table 2. The control pups were found to have an MABP of 71 ± 8 mm Hg compared to a value of 74 ± 5 mm Hg for the hemorrhagic hypotension group. Fifteen minutes following preparation, the control pups had a mean MABP of 71 ± 8 mm Hg, compared to 26 ± 4 mm Hg for the experimental group 15 minutes after insult.

Prostaglandin values are shown in Table 4. There were no significant differences in the values for 6-keto-PGF 

Values for LCGU are shown in Table 5. Control pups were found to have values of 32.1, 35.4, and 35.3 μmol/100 gm/min for frontal, temporal, and parietal cortical regions, respectively. Experimental pups were found to have values of 32.9, 36.3, and 37.0 μmol/100 gm/min for the same regions. White matter LCGU values were 16.0, 15.1, and 13.0 μmol/100 gm/min for frontal, temporal, and parietal regions of control pups compared to 19.1, 22.3, and 22.0 μmol/100 gm/min for the same regions of the hemorrhagic hypotension animals (p < 0.05 for the parietal region). No significant difference was noted in the caudate nucleus values.

**Discussion**

Newborn infants suffering from perinatal asphyxia are not uncommonly noted to have experienced episodes of fetal bradycardia and acidosis. At delivery they may be profoundly hypotensive and require vigorous resuscitation; later they may continue to demonstrate systemic evidence of ischemia-induced problems as well as persistent heart rate and blood pressure changes. In addition, CBF, believed to be pressure-passive in the severely asphyxiated newborn infant, may fall. Sankaran, et al., used jugular plethysmography to demonstrate prolonged depression of CBF in asphyxiated full-term patients, and Lou, et al., reported the loss of cerebrovascular autoregulation in asphyxiated preterm infants with respiratory distress. More recently, Volpe, et al., used positron emission tomography to demonstrate decreased CBF in parietal white matter regions in full-term neonates with asphyxia.

Consistent with animal tracer studies, full-term asphyxiated infants have been reported to show abnormalities in the parieto-occipital white matter regions of technetium brain scans, suggesting a disruption of the normal neonatal blood-brain barrier. Computerized tomography (CT) studies have demonstrated a diffuse pattern of low density consistent with edema formation throughout the cerebral hemispheres. Subsequent CT scans performed days to weeks following a perinatal asphyxial insult demonstrate parieto-occipital low-density areas and evidence of both central and cortical tissue loss.

Finally, those neonates coming to postmortem examination have been noted to have subcortical necrotic changes. These cystic lesions, also known as "periventricular leukomalacia," are believed to occur at the deep border zone of the anterior, middle, and posterior white matter regions of control pups.
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TABLE 5

Results of local cerebral glucose utilization studies
(μmol/100 gm/min)*

<table>
<thead>
<tr>
<th>Area of Observation</th>
<th>Hemorrhagic Hypotension Group</th>
<th>Control Group</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of observations</td>
<td>30</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>gray matter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>frontal</td>
<td>32.9 ± 7.7</td>
<td>32.1 ± 10.3</td>
<td>NS</td>
</tr>
<tr>
<td>temporal</td>
<td>36.3 ± 10.2</td>
<td>35.4 ± 6.1</td>
<td>NS</td>
</tr>
<tr>
<td>parietal</td>
<td>37.0 ± 6.2</td>
<td>35.3 ± 7.4</td>
<td></td>
</tr>
<tr>
<td>white matter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>frontal</td>
<td>19.1 ± 4.0</td>
<td>16.0 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>temporal</td>
<td>22.3 ± 6.2</td>
<td>15.1 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>parietal</td>
<td>22.0 ± 2.6</td>
<td>13.0 ± 4.0</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>caudate nucleus</td>
<td>23.5 ± 5.2</td>
<td>28.1 ± 6.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Mean values ± standard deviation. NS = not significant.

Although periventricular leukomalacia or neonatal stroke, postulated that these findings may offer an etiology for periventricular and occipital white matter. They observed, there was a marked decrease in CBF to the pup cerebrum, they demonstrated that, although CBF moderate hemorrhagic hypotension on the neonatal al., 53 hemorrhagic hypotension. In addition, when Young, Finegold and Michel, 7 reported significant increases exposed newborn pups to acute asphyxia evoked by the newborn puppy model. Vannucci and principle components, hypotension and hypoxia, on the newborn puppy model. Vannucci and Duffy, 46 exposed newborn pups to acute asphyxia evoked by clamping ventilatory support. They found that heart rate and systemic blood pressure fell and lactate values rose; CBF, determined by the carbon black technique, fell throughout the cerebral hemispheres.

Perinatal asphyxia in experimental animals results in both bradycardia and hypotension. 6,7,37,46 Tracer studies reveal pressure-passive CBF, the loss of normal blood-brain barrier integrity, and edema in parasagittal cerebral regions. 6,27 Edema formation may in turn result in further diminution of CBF. Although the relative roles of hypoxia, hypotension, and edema in the production of cerebral damage remains to be clarified, several workers have studied the effects of asphyxia and its principle components, hypotension and hypoxia, on the newborn puppy model. Vannucci and Duffy, exposed newborn pups to acute asphyxia evoked by clamping ventilatory support. They found that heart rate and systemic blood pressure fell and lactate values rose; CBF, determined by the carbon black technique, fell throughout the cerebral hemispheres.

Hernández, et al., documented the presence of cerebrovascular autoregulation in newborn beagle pups exposed to slow hemorrhagic hypotension. Goddard-Finegold and Michael17 reported significant increases in CBF to all brain regions in the early stages of acute hemorrhagic hypotension. In addition, when Young, et al., examined the effects of endotoxin-induced or moderate hemorrhagic hypotension on the neonatal pup cerebrum, they demonstrated that, although CBF to cortical and central gray matter structures was preserved, there was a marked decrease in CBF to the periventricular and occipital white matter. They hypothesized that these findings may offer an etiology for periventricular leukomalacia or neonatal stroke.

Similarly, Duffy, et al., examined the response of the newborn beagle puppy brain to hypoxia. Although MABP was similar in the hypoxic and the control groups of pups, marked increases were found in the CBF to brain-stem and cortical and central gray matter structures, with only small increments in CBF to the periventricular white matter of the hypoxic pups. 8 Duffy, et al., proposed that the phenomenon of the “compensatory hyperemia” may be the major homeostatic mechanism for the maintenance of oxygen availability during hypoxia. Thus, the region with the smallest increase in relative CBF would sustain the largest neuropathological deficit.

These same investigators studied the uncoupling of CBF and metabolic rate under hypoxic conditions, and found that during hypoxia an extremely high rate of glycolysis in the periventricular white matter apparently exceeds the substrate supply (that is, the CBF), such that glucose availability becomes the limiting factor for local energy production. Duffy, et al., hypothesized that this particular uncoupling of CBF and cerebral metabolic rate may be responsible for the periventricular white matter changes found in neonates with perinatal asphyxia.

These results are at variance with findings in adult animal studies, in which severe hemorrhagic hypotension causes significant decreases in both CBF and the cerebral metabolic rate of oxygen. 9,13,19,29,33,43 Although Slater, et al., reported uniformly decreased regional patterns of CBF throughout the canine brain, Chen, et al., noted a relative preservation of CBF to deep gray and brain-stem structures similar to that found in the neonatal beagle studies. 53 In addition, Savaki, et al., studied the alterations in LCGU in adult rats exposed to a model of hemorrhagic hypotension and found significant increases in glucose utilization in eight areas of the central nervous system, including brain-stem and deep gray matter structures.

These studies of hemorrhagic hypotension in the newborn beagle pup model of perinatal cerebral infarction have demonstrated significant increases in systemic PGE2 in those animals exposed to insult when compared to control pups. 18 In addition, similar to the studies of Young, et al., we found that CBF to cortical and deep gray structures was moderately preserved; in contrast, there were marked decreases in flow to temporal and parietal white matter regions. Finally, although metabolism as demonstrated by LCGU was unchanged in cortical gray matter structures, glucose utilization was significantly increased in the parietal periventricular white matter zone in our model. As in the hypoxia studies of Duffy and Cavazzuti and coworkers, this partial uncoupling of CBF and metabolism in the periventricular white matter regions may in part be responsible for the neuropathological changes found in perinatal asphyxial insult.

Acknowledgment

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Address reprint requests to: Laura R. Ment, M.D., Department of Pediatrics, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510.